

# Cryo-electron tomography on FIB-lamellae for the structural characterization of bacterial secretion systems

Citation for published version (APA):

Berger, C. (2022). *Cryo-electron tomography on FIB-lamellae for the structural characterization of bacterial secretion systems*. [Doctoral Thesis, Maastricht University]. Maastricht University. <https://doi.org/10.26481/dis.20221101cb>

## Document status and date:

Published: 01/01/2022

## DOI:

[10.26481/dis.20221101cb](https://doi.org/10.26481/dis.20221101cb)

## Document Version:

Publisher's PDF, also known as Version of record

## Please check the document version of this publication:

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## Impact

The structure of proteins and other macromolecules has been studied for decades now at high resolution using techniques such as X-ray crystallography and nuclear magnetic resonance spectroscopy (NMR). This resulted into important insights into the relationship between structure and function. However, a limitation of these techniques is that protein structure is observed in isolation, rather than the complex environment of the cells, where it performs these functions in conjunction with other proteins. Understanding how protein structure affects functions is crucial to understand their role in the cell in health in disease. Processes in the human body, such as controlling cell division and the immune system may malfunction, resulting in pathologies such as cancer and auto-immune diseases. By better understanding these processes at a structural level, more avenues are created to cure or even prevent disease. Likewise, by understanding how the structure of proteins determines how infectious pathogens, such as Tuberculosis and COVID-19, spread and cause disease can create opportunities to design better vaccines and treatments. Proteins perform complex functions at a molecular level such as manipulating other small molecules, or act as molecular motors. In the long term, a thorough understanding of how protein structure controls the function of proteins may benefit the rational design of novel proteins for biotechnological applications.

Developments in cryo-electron microscopy combined with a relatively novel method to create  $\pm 200$  nm thin slices of cells using a focused ion beam allow for a unique opportunity to study the variability of protein structure in relationship to its cellular environment. While these methods are still in an early stage, important novel scientific insights are already being obtained by its application. In this thesis project we applied and further developed these methods to study the structure of bacterial secretion systems during intracellular infection. We determined the structure of the bacterial type III secretion system of the pathogen *Yersinia enterocolitica* during intracellular infection in collaboration with scientist from other research groups, and shared these findings with the scientific community in a scientific journal. We combined observation of the structure of mycobacterial encapsulins in the cell with high-resolution structural data of isolated encapsulins to gain insight into the structural dynamics of mycobacterial encapsulin assembly and disassembly, for which a manuscript is in preparation.

We developed a method to distribute gold fiducials in cryo-FIB lamellae of cellular samples by using endocytosis. To disseminate this methodology among other scientist, the results were published in a scientific journal. For rapidly freezing cells on electron microscopy sample carriers, a novel technique that uses jets of liquid ethane to increase the cooling rate. M4i Nanoscopy and the company Cryosol (that originates from M4i Nanoscopy) are together developing a commercial instrument for jet-freezing mammalian cells. We also used our experience to write a literature review on the methodology and the scientific results obtained with them thus far, and the potential for the technique in the future, for which a manuscript for publication is in preparation. The skills and knowledge obtained on cellular cryo-electron tomography by all the scientists directly involved are invaluable for new research projects at the M4i Nanoscopy, and other research groups.